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Evolutionary and ecological implications of genome size in the North American endemic sagebrushes (subgenus *Tridentatae*, *Artemisia*, Asteraceae)[1]

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Genome size of 48 populations belonging to the North American sagebrushes and related species (mainly from subgenus *Tridentatae* of the genus *Artemisia*) has been assessed by flow cytometry. The higher monoploid genome size of the *Tridentatae* compared with that of the other subgenera of *Artemisia* is confirmed. The possibility of finding any connection between genome size data and different traits of the studied species was examined. Little interspecific variation was found. Such homogeneity in nuclear DNA amount (together with the high morphological, chemical and karyological affinities) supports the hypothesis of a recent diversification process in this restricted group. This fact, the incongruence detected in other studies including ITS or cpDNA phylogenies and the natural tendency of these species to hybridize point to a likely reticulate evolution. The most notable difference in 2C-value was found in *A. pygmaea* and on the basis of its morphology, ecology and higher genome size, it is hypothesized a derived, more specialised condition for the pigmy sagebrush. Intraspecific nuclear genome size variation has also been assessed, and low values were observed in most cases. Additionally, genome size of the hybrids analysed in this study is close to the expected mean of that of parental species.

Key words: C-value; Compositae; hybridization; interspecific variation; North America; r-K strategies; reticulate evolution; speciation.

The *Tridentatae*, one of the most ecologically and economically important subgenera of the genus *Artemisia* (Asteraceae, Anthemideae), comprise 11 species (and about 20 taxa all together including subspecific entities) of landscape-dominant, xerophytic shrubs, endemic to North America (McArthur et al., 1981, McArthur and Sanderson, 1999). Apart from their distribution, they differ from the rest of *Artemisia* s. l. by their completely woody nature, distinctive chemistry, molecular genetics and fertile, homogamous, perfect disc flowers (McArthur, 1979; McArthur and Sanderson, 1999; Torrell et al., 1999). Chromosome counts confirm the base chromosome number x = 9, with ploidy levels ranging from 2x to 8x, but mostly 2x (48%) and 4x (46%) (McArthur and Sanderson, 1999). Polyploidy occurs in nine out of the 11 species and in many subspecies as well (McArthur and Sanderson, 1999). Based on evidence from different sources, such as ITS sequences of nuclear ribosomal DNA or chloroplast DNA restriction site data, among others, the *Tridentatae* can be considered a monophyletic group (Kornkven, Watson and Estes, 1998; Torrell et al., 1999; Vallès et al., 2003).

The origin of the *Tridentatae* is not resolved although two main hypotheses are extant. The available data (molecular, karyotypical, chemical and morphological) favour one hypothesis or the other (Rydberg, 1916; Ward, 1953; McArthur and Plummer, 1978, Bremer and Humphries, 1993; Ling, 1995a, 1995b). The first one, supported by Ling (1991a, 1991b) and Bremer and Humphries (1993), maintains that these plants originated from the Eurasian *Seriphidium* that migrated over the Bering Strait, while McArthur et al. (1981) acknowledge an Eurasian origin for the genus but suggest that the *Tridentatae* evolved from herbaceous members of subgenus *Artemisia* in North America. Subgenus *Artemisia* species currently occur in eastern Siberia and Alaska (the Bering Strait area)

whereas subgenus *Tridentatae* and subgenus *Seriphicium* plants do not (McArthur and Plummer, 1979). We believe that the nascent *Tridentatae* differentiated there under the stimulus of recurring aridic climatic cycles that took place during the Pleistocene.

The most abundant and widespread species from this subgenus is *A. tridentata* Nutt. including its five subspecies (three more common: *A. tridentata* subsp. *tridentata* Nutt., *A. tridentata* subsp. *vaseyana* (Rydb.) Beetle and *A. tridentata* subsp. *wyomingensis* Beetle & A. L. Young, and two less common: *A. tridentata* subsp. *spiciformis* (Osterh.) Kartesz & Gandhi, and *A. tridentata* subsp. *xericensis* Winward). In fact, the subgenus can be considered a large species complex (Clausen, 1951) centered on *A. tridentata*, because hybridization between taxa at all levels appears to be possible (McArthur et al., 1979; McArthur et al., 1988). Some other species are ecologically important as well and are also landscape-dominant, such as *A. arbuscula* Nutt., *A. cana* Pursh, and *A. nova* A. Nelson. The rest of the *Tridentatae* (*A. argillosa* Beetle, *A. bigelovii* A. Gray, *A. longiloba* (Osterh.) Beetle, *A. pygmaea* A. Gray, *A. rigida* A. Gray, *A. rothrockii* A. Gray, and *A. tripartita* Rydb.) are more restricted in distribution.

Genome size has recently received more attention, in many plant research fields e. g. ecology, plant distribution, systematics, taxonomy. Since the 1950's, when the term C-value was coined by Swift (1950), much research has increased the knowledge of plant C-values (Bennett and Leitch, 2004, 2005a, 2005b). Studies have shown huge differences in DNA amounts between various kinds of organisms, a topic known as genome size enigma (Gregory 2001, 2005). Molecular mechanisms that can lead to an increase or a decrease in genome size have been extensively studied (Petrov et al., 2000; Bennetzen et al., 2005). Relationships between genome size and cytological traits, reproductive biology, ecology, environment features, geographic distribution, biomass production and many other plant

characteristics have been investigated (and established) by the scientific community in recent years (Graham, Nickell and Rayburn, 1994; Poggio et al., 1998; Ohri, 2005). Also, the possibility of genome size variation (at species or subspecific levels) has been studied and debated (Greilhuber, 2005; Murray, 2005).

The interesting data that can be extracted from genome size analysis stimulated the study on this subject in the *Tridentatae* with these objectives: a) to exploit the nuclear DNA amount information for taxonomic purposes, that is, to identify evolutionary relationships between these plants together with the scarce phylogenetic data existing for them (see Kornkven et al., 1998; Torrell et al., 1999; Watson et al., 2002; Vallès et al., 2003); b) to detect any relationship between the nuclear DNA amounts and traits of these plants (plant size, resistance to fire, growth rate), their surrounding environmental features (mean annual precipitation, aridity, altitudinal range), and geographical distribution (local or widespread), abundance and competitive nature; c) to study the scope, if any, of the intraspecific and interspecific genome size variation in the *Tridentatae*; and, finally, d) to increase general knowledge in *Artemisia* C-values and, particularly, to complete the survey of the *Tridentatae* 2C values, which is, to date, limited (Torrell and Vallès, 2001; Garcia et al., 2004).

MATERIALS AND METHODS

Plant material--- Table 1 shows the 48 populations studied, along with their site of origin and collection information. Eleven *Tridentatae* species, with 14 subspecific entities, four populations of two hybrids and five closely related *Artemisia* species have been investigated. The selected populations represent distinct geographic areas, ploidy levels (from diploid to octaploid) and chromosome numbers. Vouchers are

deposited in the herbarium of the Shrub Sciences Laboratory, Provo, UT (SSLP). In addition to a complete representation of the *Tridentatae* as accepted by McArthur (McArthur and Sanderson, 1999; McArthur, 2005), we have also included *A. californica* Less., *A. filifolia* Torr., *A. ludoviciana* Nutt., *A. palmeri* A. Gray, and *A. papposa* S. F. Blake & Cronquist. These species were included for comparative purposes for this study and a companion molecular genetic study (S. Garcia et al., Universitat de Barcelona, unpublished data). All are North American species that are sympatric or tightly parapatric with representatives of the *Tridentatae. Artemisia palmeri* is a large woody plant endemic to the coastal area near San Diego, California. It has been treated as a member of the the subgenus *Seriphidium* (Ward, 1953) and also considered in an independent genus, *Artemisiastrum* (Rydberg, 1916). It has the floral formula of no ray flowers and fertile hermaphrodic disc flowers, the *Seriphidium/Tridentatae* formula (McArthur 1979). However, it is disjunct from other *Seriphidium,* an otherwise Eurasian group, and displays a growth and leaf form, albeit on a woody macro scale, reminiscent of its subgenus *Artemisia* (especially *A. ludoviciana*) congeners (Shultz, 1993). Another species, *A. californica*, belongs to the subgenus *Artemisia.* However, it is woody unlike most subgenus *Artemisia* species (Hall and Clements, 1923). *Artemisia filifolia* is from the subgenus *Dracunculus*. *Artemisia californica* and *A. filifolia* grossly resemble one another in that they are of similar size with a willowly, filiform appearance and both share cpDNA segments with the *Tridentatae* (Kornkven et al., 1999). *Artemisia filifolia* also has other affinities with the *Tridentatae*–a similar karyotype (McArthur and Pope, 1979) and similarities in secondary chemistry (Kelsey and Shafizadeh, 1979).

Young leaves used for flow cytometry assays were taken from plants cultivated in pots, either from previous achene germination in Petri dishes or from adult plant transplantation. The achenes or adult plants were collected from natural populations or in the case of synthethic hybrids from cultivated plants. Seeds of *Pisum sativum* L. cv. Express long and of *Petunia hybrida* Vilm. cv. PxPc6, both used as internal standards for flow cytometric measurements, were obtained from the Institut des Sciences du Végétal (CNRS, Gif-sur-Yvette, France).

Flow cytometry measurements— DNA 2C-values of the tested species were estimated using flow cytometry. *Pisum sativum* L. cv. Express long and *Petunia hybrida* Vilm. cv. PxPc6 (2C=8.37 pg and 2.85 pg, respectively, Marie and Brown, 1993) were used as internal standards to cover the range of 2C-values

found. Young healthy leaf tissue from the species being studied and a calibration standard were placed together in a plastic Petri dish and chopped in Galbraith's isolation buffer (Galbraith et al., 1983), with a razor blade. The amount of target species leaf (about 25 mm^2) was approximately twice that of the internal standard. The suspension of nuclei in the isolation buffer was filtered through a nylon mesh with a pore size of 30 μm, supplemented with 100 μg/mL ribonuclease A (RNase A, *Boehringer*, Meylan, France) and stained for 20 minutes with propidium iodide (Sigma-Aldrich Química, Alcobendas, Madrid, 60 μg/mL), the chosen fluorochrome (Johnston et al., 1999); tubes were kept on ice during staining and then left at room temperature until measurement. For each population, five individuals were analyzed; two samples of each individual were extracted and measured independently. Measurements were made at the Serveis Cientificotècnics generals de la Universitat de Barcelona using an Epics XL flow cytometer (Coulter Corporation, Hialeah, Florida). The instrument was set up with the standard configuration: excitation of the sample was done using a standard 488-nm air-cooled argon-ion laser at 15 mW power. Forward scatter (FSC), side scatter (SSC), and red (620 nm) fluorescence for propidium iodide were adquired. Optical alignment was based on optimized signal from 10-nm fluorescent beads (Immunocheck, Epics Division, Coulter Corporation, Hialeah, Florida). Time was used as a control of the stability of the instrument. Red fluorescence was projected on 1,024 monoparametrical histogram. Gating single cells by their area versus peak fluorescence signal excluded aggregates. Acquisition was automatically stopped at 8000 nuclei. The total nuclear DNA content was calculated multiplying the known DNA content in *Pisum* or *Petunia* by the quotient between the 2C peak positions of *Artemisia* and the chosen internal standard in the histogram of fluorescence intensities for the 10 runs, and mean values and standard deviations were calculated based on individual plants; on the assumption that there is a linear correlation between the fluorescence signals from stained nuclei of the unknown specimen and the known internal standard and the DNA amount.

Statistical analyses--- Analysis of variance, means comparison by least significant difference test, LSD, and simple regression were carried out to evaluate the relationships between the studied variables. All the analyses were performed with the program Statgraphics Plus 5.0 (Statistical Graphics Corp., Rockville, Maryland). In addition to the data obtained in the present study, *Tridentatae* data from prior papers on

Artemisia genome size (Torrell and Vallès, 2001; Garcia et al., 2004) were integrated into the statistical analyses of the present study.

RESULTS

Table 2 gives 2C DNA amounts estimated for the sampled taxa (together with other karyological data such as ploidy level and chromosome number, as well as genome size data in megabase-pairs). 2C values ranged from 7.26 pg in *A. filifolia* (diploid species) to 27.04 pg in *A. cana* subsp. *cana* (octaploid), and monoploid genome sizes from 3.38 pg in *A. cana* subsp. *cana* to 5.57 pg in *A. pygmaea* (a range of variation of 37.24% and 16.48% in each case). The analyses were of good quality (HPCV= 1.96%). This is our fourth study on *Artemisia* nuclear DNA amounts by flow cytometry, but the first especially focused on the subgenus *Tridentatae*. For *A. longiloba*, *A. ludoviciana*, *A. papposa*, *A. rigida*, *A. tripartita* and for two subspecies of *A. tridentata* (*A. tridentata* subsp. *wyomingensis* and *A. tridentata* subsp. *xericensis*), the present work contributes the first estimation of their nuclear DNA amounts. For the remaining taxa we had already given other estimations (see Torrell and Vallès, 2001 and Garcia et al., 2004 for details). With the present data, genome sizes are known for all the species and subspecies of the subgenus *Tridentatae*.

DISCUSSION

***Interspecific genome size variation within true sagebrushes (subgenus* Tridentatae)**—* Just a glance to the data on genome sizes for these species leads us to a conspicuous conclusion: within each of the ploidy levels studied, similar nuclear DNA amounts for the different species are obtained. With the exception of *A. pygmaea*, an unusual taxon which we will discuss in more detail later, all the other diploid *Tridentatae* show a comparable nuclear DNA amount (range, 8.17 to 9.47 pg). Even non-*Tridentatae Artemisia* included in this study fall within or are close to this range. The same conclusion can be reached when examining the results for each of the ploidy levels studied. Contrary to this finding, some authors have reported meaningful differences in genome sizes on other data sets between taxa belonging to the same narrow group of species, but others support our results (see Ellul et al., 2002; Garnatje et al., 2004; Chase et al., 2005; Garcia et al., 2005). Additionally, a lack of substantial morphological variation at the interspecific level, particularly the absence of discriminating morphological characters with systematic value, has created difficulties, not only in evaluating phylogenetic relationships within the *Tridentatae*, but also with the circumscription of the subgenus (Cronquist, 1994; Kornkven et al., 1998; Kornkven et al., 1999; Watson et al., 2002; Shultz, in press).

However, a deep re-examination of the data shows that genome sizes of these species, though very similar for each ploidy level, exhibit a continuous variation "fashion", in contrast to the observations in other plant groups whose nuclear DNA amounts seem to jump, in a quantum variation "fashion", from one species to another

closely related species (Garnatje et al., in press –genus *Carthamus*-; Garcia et al., in press a –subgenus *Absinthium*). Quantum shifts in genome size have been detected within plants and algae (Narayan 1985, 1988, 1998; Maszewski and Kolodziejczyk, 1991). Similarly to our findings, Ellul et al. (2002) found in *Cistus* a mainly continuous variation trend in DNA content, with overlapping values among sectional and even subgeneric taxa. This continuous variation might point to a process of reticulate evolution, in which species evolve by various modes of hybridization and backcrossing. This probably slows the tempo of evolutionary change. Additionally, their restricted morphological differences may be a reflection of limited genetic differences, which indeed would mean that there are no "genetical barriers" for them to interbreed, this fact enhancing their "promiscuity". Given that it is likely that they share very similar genomes, which also favours hybridization, this obstructs (if not inhibits) the establishment of a more robust phylogenetic framework for these species, due to the lack of genetic regions showing enough mutational rate to generate substantive differences between the taxa. Indeed earlier studies demonstrated a uniform karyotype in the *Tridentatae* (McArthur et al., 1981) and low levels of genetic divergence in nuclear ITS and plastid intronic and intergenic sequences in the *trn*L region (Stanton et al., 2002). Moreover, if they occupy similar habitats they have had no need of differentiating, and only those species colonizing new habitats would have adaptated to them. In fact, the process would have been just the opposite, namely, those taxa which had differentiated genomes would be the ones able to adapt to new conditions and habitats.

The statistical analyses performed show significant differences ($p=0.0291$) between the monoploid genome size of the *Tridentatae* with respect to that of the non-

Tridentatae Artemisia, with the *Tridentatae* genome size being the largest. The present findings agree with those of Torrell et al. (1999, 2003) and Vallès et al. (2003), who showed the independence of the subgenera *Tridentatae* and *Seriphidium* on the basis of the analysis of the nuclear ribosomal DNA ITS sequences and of molecular cytogenetic markers. Moreover, and to avoid the bias produced due to comparisons between species of different ploidy levels, when diploid *Tridentatae* and diploid non-*Tridentatae Artemisia* 2C values are compared, the analysis of variance also shows that nuclear DNA amount of the former is significantly higher ($p=0.008$) than that of the remaining groups. As previously reported in Garcia et al. (2004) this also supports the standing of the true sagebrushes as an independent group in the genus *Artemisia*, well differentiated from the other subgenera. Additionally, as numerous previous studies in many other plant genera have stated (Leitch and Bennett, 2004, and references therein) differences in monoploid genome size between each ploidy level are also statistically significant ($p=0.008$), hence a decreasing monoploid genome size is detected with increasing ploidy levels, confirming again the loss of nuclear DNA during the process of polyploidization.

This increased genome size of the *Tridentatae* in comparison to their relatives of the genus *Artemisia* could be related with the fact that this group of species, colonizers of extensive areas of which they are landscape-dominant, are therefore subject reduced competitive pressures. That is, the adaptation to that particular environment is so good in the sagebrushes that competitors are at a disadvantage. Is this why they can loosely expand their genome size? One of the most outstanding mechanisms that might explain an increase in genome size, apart from the relevancy of transposable elements activity which is recently receiving more interest in the study of genome size (see Kellogg and

Bennetzen, 2004), is the well-known process of polyploidization. Actually, the *Tridentatae* tend to polyploidize easily, e.g. 2/3 of the population of *A. nova* are tetraploids and 1/3 diploids, and the populations of *A. rothrockii* or *A. argillosa* are entirely polyploid (McArthur and Sanderson, 1999, Mahalovich and McArthur, 2004). Polyploidy has been linked to a better adaptation to extreme environments (Hagerup, 1932), because polyploids have greater heterozygosity and biochemical diversity than diploids, so they might have broader ecological tolerances than do diploids. Were this the case, polyploids would tend to occur in more geographical regions and in more habitats within regions than diploids (Levin, 2002). Similarly, Ehrendorfer (1980) and Stebbins (1985) contend that relatively young polyploids tend to occur in disturbed or seral communities, whereas older polyploids are expected in more stable or climax communities. The extensive desertic areas of North America could be such an extreme environment, so this could explain their tendency to multiply their genome (Sanderson, McArthur and Stutz, 1989; McArthur et al., 1998; McArthur and Sanderson, 1999). In fact, in the genus *Artemisia*, many of the species colonizing extremely arid lands are polyploid (Garcia et al., in press b), supporting the hypothesis that there is a connection between ecological tolerance and polyploidy in many plant groups (Otto & Whitton, 2000). However, it is yet not clear whether polyploidy (or a higher C-value) is the cause or the consequence of the *Tridentatae*'s optimal adaptation. On the other hand, there is the case of many island colonizers species, where a significant reduction in genome size has been detected in response to insular selection pressures (Suda, et al., 2003; Garcia et al., in press a; T. Garnatje et al., Institut Botànic de Barcelona, submitted). On the basis of these observations it is hypothesized that in absence of any competition pressure acting on the plants, the genome can (and probably tends to) expand itself, while in environments subject to competitive constraints,

the trend is the decrease in total nuclear DNA amounts. This fact could also be linked with the environmental abundance or lack of any important nutrient for the DNA synthesys, such as phosphorus – basic constituent of the double helix-, which could allow or inhibit genome size expansion (A. Leitch, University of London, pers. comm.).

Intraspecific variation--- Variation in DNA amount between species begins with changes within species, yet intraspecific variation remains one of the most controversial topics in the study of plant genome size (Bennett and Leitch, 2005). In the *Tridentatae*, a low interspecific variation is also accompanied by a low intraspecific variation at the same ploidy level, which reaffirms the homogeneity in genome sizes within this group. Up to now, the extent of intraspecific genome size variation is hotly debated (Greilhuber, 1998, 2005) and some authors attribute such variation to methodological errors or taxa misidentification (Greilhuber, 1998; Ohri, 1998). However, factors like changes in repetitive DNA (Rabinowicz, 2000) or retrotransposon activity (Bennetzen et al., 2005) can be a source of variation within a species. Among the different populations of the same taxon which have been assessed in this study and in the previous ones (Torrell and Vallès, 2001; Garcia et al., 2004; Garcia et al., in press a), low percentages of intraspecific variation have been detected for the majority of species (the most around 1 to 2%). Even in the case of a segregating population of *A. tridentata* subsp. *parishii*, which have clearly distinct flowering phenotypes (McArthur, 2005), one being upright and the other droopy (collections 3037 and 3038, respectively, of Table 2), differences in nuclear DNA amounts between them are negligible. We suspect the flowering phenotypes are gene differences, not genomic ones. However, morphological differences do not necessarily imply changes in the genome sizes, as this finding confirms. On the other hand, a considerable variation

has been found in *A. pygmaea*, with a 5.98% and in *A. tridentata* subsp. *spiciformis*, up to a 10.02% (Table 2). Doležel and Bartoš (2005) state that a 5% of variation should be considered as acceptable in some groups; methodological error could have probably been the cause of the variation detected in these species.

Hybrid formation— Some studies have reported that diploid interspecific hybrids have an intermediate DNA content between values of the parents involved (Buitendijk et al., 1997). As it has been previously mentioned, owing to their widespread and intensive distribution *Tridentatae* taxa overlap substantially and they tend to hybridize (McArthur et al., 1979, 1988). Hybridization is one of the natural evolutionary mechanisms in the sagebrushes together with polyploid formation as in many other plant groups (Ward, 1953; McArthur et al., 1981,1988; McArthur and Sanderson, 1999). Hybridization is favoured by the existence of those overlapping areas, by their wind-pollinated nature and probably by a similarity between their respective genomes which is another sign of their recent diversification process. The data set in this study includes genome sizes for two hybrids: a diploid and a tetraploid *A. tridentata* subsp. *tridentata* x *A. tridentata* subsp. *vaseyana*, and two populations of an hexaploid *A. cana* subsp. *cana* x *A. tridentata* subsp. *wyomingensis* (these hexaploids are a result of a the hybrid combination of octaploid *A. cana* subsp. *cana* being hybridized with tetraploid *A. tridentata* subsp. *wyomingensis*—see McArthur et al., 1998 and McArthur and Sanderson, 1999). The hybrid combination between the two subspecies of *A. tridentata* produced both diploid and tetraploid plants (Table 2). The tetraploid plant (collection 3048 in Tables 1 and 2) is apparently a spontaneous tetraploid; a circumstance not unexpected in a group that generates polyploidy-see McArthur et al., 1998 and McArthur and Sanderson, 1999. The nuclear DNA amounts of the hybrids are

consistent with the expected means corresponding to their parents' genome sizes (Figure 1), although the tetraploid and hexaploid hybrids shows a little less than that mean, probably due to its polyploid nature (and the diminution that tends to accompany the process of polyploidy, as previously cited).

Genome size variation, morphology and environment--- We have tried to detect any significant relationship between genome size and some traits of the species studied. The data has been extracted from the abundant literature existing for the *Tridentatae* and allies (McArthur et al., 1979; Cronquist, 1994; McArthur and Stevens, 2004; Shultz, in press), as sagebrushes have been studied in North America from many different points of view due to their important ecological and economic role. The characteristics evaluated were: altitude, altitude range (narrow or wide), capacity of recover after burning, distribution (local or widespread), drought tolerance, growth rate, mean annual precipitation, mean plant height, salinity tolerance, seed production and vegetative growth (absence or presence). Only diploid taxa have been used for the analysis, to avoid biased results due to monoploid genome downsizing in polyploids. A summary of the evaluated characteristics are presented in Table 4. No significance has been observed in most cases, which is not surprising given the homogeneity of results at the same ploidy level. The analysis of regression with altitude was not significant ($p<0.05$). This notwithstanding, some statistically significant relationships were detected: species with higher C-values tend to inhabit areas with lower mean annual precipitation ($p=0.0405$), narrower altitude range ($p=0.0068$) and lower seed production ($p=0.0025$); indeed, a lower seed production is correlated with plants inhabiting desertic areas. However, a trend is outlined intuitively from these relationships and by observation of the data: it seems that slow-growing, few

seed producers, more drought tolerant and smaller species tend to have higher DNA amounts whereas fast-growing, high seed producers, less drought tolerant and bigger ones tend to show lower nuclear DNA amounts.

***Taxa of doubtful taxonomic position and genome size. The particular case of* A. pygmaea**— Sugbenus *Tridentatae* still remains unresolved from the phylogenetic point of view, as it has yet been mentioned. Although the monophyly of this group is strongly supported by different studies, such as ITS and cpDNA phylogenies (Kornkven et al., 1998), ITS and ETS (Torrell et al., 1999; Vallès et al., 2003; M. Sanz et al., Universitat de Barcelona, unpublished data), some anomalous species have had their position within the *Tridentatae* questioned. It is in these cases where a critical study of their nuclear DNA amount can contribute to the clarification of their taxonomic placement. Inter and intraspecific C-value variation in a narrow group of species has been considered as a predictor of taxonomic heterogeneity and as an indicator of speciation in process (Murray, 2005), and consequently genome size study reveals itself as a useful tool in elucidating evolutionary relationships within tightly related taxa.

Artemisia bigelovii – a taxon that has been placed in and out of the subgenus *Tridentatae* based on floral morphology, karyotype, and molecular genetic data (Ward, 1953; McArthur et al., 1981; Kornkven et al., 1998; Shultz, in press) –, shows a nuclear DNA amount fairly similar to the other *Tridentatae* (of the same ploidy level). Bigelow sagebrush has been considered to occupy an intermediate position between the true sagebrushes and subgenus *Artemisia*, although generally treated as a *Tridentatae* on the basis of many characters such as wood anatomy, leaf form, karyotype, RAPD genetic

markers and analysis of ITS sequences in nuclear ribosomal DNA (Kornkven et al., 1998; McArthur et al., 1981, 1998). Also, its nuclear DNA amount supports the inclusion of this species in the subgenus.

On the other hand, a doubtful taxonomic position can be well reflected in genome size. *Artemisia pygmaea* has an uncertain placement within the *Tridentatae*. Compared with other *Tridentatae* species, pygmy sagebrush is a dwarf, depressed shrub, with different leaf morphology and larger seeds (Cronquist, 1994; McArthur and Stevens, 2004) than the rest of the group. It is relatively uncommon and occurs on dry alkaline sites where few other species can survive, probably due to its numerous morphological adaptations to these extremely xeric sites which tends to inhabit. Nonetheless, it has the karyotypic and molecular characteristics of *Tridentatae*, and balance of evidence has favoured its inclusion within the true sagebrushes (McArthur et al., 1981 and references therein). However, some molecular biology studies place this species as sister to the other *Tridentatae* (Kornkven et al., 1998; Watson et al., 2002). A cluster analysis using nuclear DNA amounts has also placed *A. pygmaea* isolated from all the other diploid *Tridentatae* of this study (Figure 2). In fact, genome size of *A. pygmaea* (2C=11.19 pg) is, by far, the highest of all the other *Tridentatae* diploids studied (which range from 8.17 to 9.47 pg).

On the basis of its specialized morphology and higher genome size, it is feasible that *A. pygmaea* occupied a derived position within its group, bearing in mind that ancestral species are supposed to have lower DNA amounts and derived ones own higher genome sizes (Leitch et al., 1998). Taking up again the finding that subgenus *Tridentatae* showed an increased genome size with respect to the other subgenera of *Artemisia*, and the particularly outstanding case of *A. pygmaea*, it is suggested that this could be related with

the change in the colonizing strategy of the genus, from the r to the K-strategy, particularly in some species of the *Tridentatae*. As McArthur and Plummer (1978) and McArthur et al. (1981) had previoulsy speculated, it is likely that some species of subgenus *Artemisia* from central Asia were the ancestors of the present *Tridentatae*. Species of this subgenus widely distributed in North America include *A. frigida* and *A. ludoviciana*, among others. Of particular interest is the fringed sage, *A. frigida*, since it is a continent bridging with a natural geographical range embracing west-central North America, Mexico, Siberia and central Asia. Its low DNA content (5.25 pg, Garcia et al., 2004) suggests a possible role as ancestral stock for the *Tridentatae*. Additionally, the 2C value for the tetraploid *A. ludoviciana* analysed in this study (2C=13.82 pg) is quite low in comparison with the tetraploid *Tridentatae* studied (it has not been possible to analyse a diploid population of *A. ludoviciana*, but a lower monoploid genome size around 7 pg can be deduced from this value). This species and relatives tend to show higher seed production, faster cell cycles, extensive distribution and lower genome sizes, namely they probably follow the r-strategy. Conversely, some of the *Tridentatae*, and particularly *A. pygmaea,* show a more restricted distribution, slower cell cycle and bigger seeds, which fits better with the K-strategy (a given species will mainly adopt one strategy, even though shared treats of the other strategy should not be overlooked). Given that the K-strategy is considered as more progressive in an evolutionary sense (Margalef, 1974; Harper, 1977), this fact would also point out the more derived condition of the *Tridentatae*.

Finally, this study has also reported genome size data for some *Artemisia* species sympatric or tightly parapatric to the *Tridentatae* but which clearly belong to other sections in the genus: *Artemisia papposa* and *A. californica*, with respectively 8.04 and

8.48 pg, are quite similar in terms of genome size to the *Tridentatae*; however, *A. filifolia* (subgenus *Dracunculus*) and *A. palmeri* (treated by different authors in the subgenera *Seriphidium* or *Artemisia*, or also in an independenent genus, *Artemisiastrum*) show a noticeably lower genome sizes than do their *Tridentatae* counterparts.

In summary, this study is the first performed on nuclear DNA amounts in an extensive sample of the *Tridentatae* and some other *Artemisia* species that are sympatric and may be related. From the homogeneity of most of the results, we can conclude that the North American sagebrushes form a homogeneous group, with differentiated characteristics from the rest of *Artemisia* species and which are probably undergoing a diversification and speciation process but have not yet differentiated C-values between the majority of these taxa in a same ploidy level. Additionally, this homogeneity points to a process of reticulated evolution, a hypothesis reinforced by the difficulty (and incongruences) of many authors to establish a clear phylogenetic framework for the *Tridentatae*. A change in the colonizing strategy within the North American *Artemisia* and an advanced position for the pygmy sagebrush is suggested on the basis of genome size data. It is likely that when *Artemisia* species arrived to North America they needed the r-strategy in order to colonize the new territories but there are some taxa, such as *A. pygmaea*, which represent the particular need of surviving in a specially difficult and concrete environment, and possibly follow the K-strategy. Its increased genome size could be a consequence of a need of more gene copies: while one copy maintains the usual function, the other(s) evolve until the acquisition of a new function which allows some kind of advantageous adaptation. In the long term, when the new function is fixed, this excess of DNA will probably be eliminated.

Finally, in order to achieve a better understanding of their genome size variation, there is a need of elucidating the complex systematic relationships among the *Tridentatae*, given that the results obtained in this field are still not conclusive. Molecular cytogenetic studies (started in the paper by Torrell et al., 2003, and followed by S. Garcia et al., Universitat de Barcelona, unpublished data) could contribute to clarify the relationships between the *Tridentatae* and the species related to them.

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TABLE 1. Provenance of the populations of *Artemisia* studied.

Taxa	Origin of materials	Collection number[1]
Tridentatae		
Artemisia arbuscula subsp. *arbuscula*	Corn Creek Canyon, Millard Co. Utah. 1830 m.	2877
A. arbuscula subsp. *arbuscula*	South of Jordanelle Reservoir, Wasatch Co., Utah. 1890 m.	3027
A. arbuscula subsp. *arbuscula*	Sage Junction, Lincoln Co., Wyoming. 1930 m.	3028
A. arbuscula subsp. *longicaulis*	Toulon, Pershing Co. Nevada. 1335 m.	2860
A. arbuscula subsp. *longicaulis*	Bruneau, Owyhee Co. Idaho. 1012 m.	2855
A. arbuscula subsp. *termophila*	East bank of Snake River, Yellowstone National Park, Teton Co., Wyoming. 2093 m.	3032
A. argillosa	Coalmont, Jackson Co., Colorado. 3497 m.	3034
A. bigelovii	Emery Co. Utah. 1801 m.	2869
A. bigelovii	15 km east of Fremont Junction. Emery Co. Utah. 1777 m.	3050
A. bigelovii	Padre Canyon, Cocconino Co., Arizona. 1799 m.	3051
A. cana subsp. *bolanderi*	17 km northwest of Bridgeport, Mono Co., California. 2270 m.	3047
A. cana subsp. *cana*	Sheridan, Sheridan Co. Wyoming. 1140 m.	2128
A. cana subsp. *viscidula*	Strawberry Valley, Wasatch Co. Utah. 2374 m.	2844

A. cana subsp. *viscidula*	Soldier Summit, Wasatch. Co.	
2875		
	Utah. 2255 m.	
A. cana subsp. *viscidula*	Fossil Butte National Monument,	2851
	Lincoln Co. Wyoming, 1650 m.	
A. cana subsp. *cana* x *A. tridentata*	Pleasant Grove Plots, Uinta National	2759
subsp. *wyomingensis*[2]	Forest, Utah Co., Utah. 1734 m.	
A. cana subsp. cana x *A. tridentata*	Pleasant Grove Plots, Uinta National	2760
subsp. *wyomingensis*[2]	Forest, Utah Co., Utah. 1734 m.	
A. longiloba	Evanston, Uinta Co., Wyoming. 2067 m.	3025
A. nova	Tunnel Spring, Desert Experimental Range.	2876
	Millard Co. Utah. 2174 m.	
A. nova	Pine Valley Pass, Millard Co.	2873
	Utah. 1820 m.	
A. nova	Birch Springs Road, Mount Borah,	3053
	Custer Co., Idaho. 2120 m.	
A. nova var. *duchesnicola*	Tridell Road, Uintah Co., Utah. 1702 m.	3029
A. nova var. *duchesnicola*	Tridell Road, Uintah Co., Utah. 1702 m.	3030
A. pygmaea	Yuba Dam Road, Juab Co. Utah. 1535 m.	2870
A. pygmaea	San Rafael Swell, Emery Co.	2836
	Utah. 2195 m.	
A. rigida	Malheur Reservoir, Malheur Co.	2859
	Oregon. 1035 m.	
A. rothrockii	Reed Flats, White Mountains,	19803[3]
	Inyo Co., California. 3072 m.	
A. tridentata subsp. *parishii*[4]	West of Rosamond, Kern Co., California. 722 m.	3037
A. tridentata subsp. *parishii*[4]	West of Rosamond, Kern Co., California. 722 m.	3038
A. tridentata subsp. *spiciformis*	Ford Ridge, Bristle Cone Scout Camp,	2839

	Carbon Co. Utah. 2856 m.	
A. tridentata subsp. *tridentata*	Salt Cave Hollow, Salt Creek Canyon, Juab Co. Utah. 1870 m.	2871
A. tridentata subsp. *tridentata*	Beaver, Beaver Co. Utah. 1780 m	McArthur, s. n.
A. tridentata subsp. *tridentata* x *A. tridentata* subsp. *vaseyana*	McArthur's back yard. Orem, Utah Co., Utah. 1474 m.	3049
A. tridentata subsp. *tridentata* x *A. tridentata* subsp. *vaseyana*	Grounds of the Shrub Sciences Laboratory. Provo, Utah. 1374 m.	3048
A. tridentata subsp. *vaseyana*	Salt Cave Hollow, Salt Creek Canyon, Juab Co., Utah. 18780 m.	2872
A. tridentata subsp. *vaseyana*	Hobble Creek Canyon, Utah Co. Utah. 1555 m.	2874
A. tridentata subsp. *vaseyana*	Spring City, Sanpete Co. Utah. 1950 m.	2879
A. tridentata subsp. *wyomingensis*	Gordon Creek, Carbon Co. Utah. 1980 m.	2880
A. tridentata subsp. *xericensis*	Mann Creek Reservoir, Washington Co. Idaho. 929 m.	2858
A. tripartita subsp. *rupicola*	Pole Mountain, Albany Co., Wyoming. 2647 m.	3033
A. tripartita subsp. *tripartita*[5]	Dubois Sheep Station, Clark Co. Idaho. 1650 m.	2845
A. tripartita subsp. *tripartita*	Birch Springs Road, Mount Borah, Custer Co., Idaho. 2191 m.	3054

Outgroup species

A. californica	Santa Clarita, Los Angeles Co., California. 487 m.	3039
A. californica	Los Peñasquitos Canyon Preserve, San Diego, San Diego Co., California. 70 m.	3043
A. filifolia	Moccasin, Mohave Co. Arizona. 1530 m.	2868

A. ludoviciana	Salt Cave Hollow Road, Uinta National Forest, Salt Creek Canyon, Juab Co., Utah. 2084 m.	3087
A. palmeri	Los Peñasquitos Canyon Preserve, San Diego, San Diego Co., California. 70 m.	3044
A. papposa	Milepost 130, U. S. Highway 20, 16 km west of Hill City. Elmore Co.Idaho. 1679 m.	3077

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[1] McArthur collection numbers; vouchers are deposited in the herbarium of the Rocky Mountain Research Station, Provo, Utah (SSLP).

[2] Synthetic hybrids (see McArthur et al., 1998 and McArthur and Sanderson, 1999).

[3] Collection number from Leila M. Shultz.

[4] Separate floral morphologies (see McArthur, 2005).

[5] This A. *tripartita* subsp. *tripartita* may be introgressed with *A. tridentata* subsp. *wyomingensis.*

TABLE 2. Nuclear DNA content and other karyological characters of the populations studied.

Taxa	2C ± s.d. (pg)[1]	2C (Mbp)[2]	2n[3]	Ploidy level	2C/P.l.[4]	Standard[5]
Subgenus *Tridentatae*						
Artemisia arbuscula subsp. *arbuscula* (2877)	9.21± 0.06	9007.38	18	2	4.61	*Petunia*
A. arbuscula subsp. *arbuscula* (3027)	9.04 ± 0.13	8841.12	18	2	4.52	*Petunia*
A. arbuscula subsp. *arbuscula* (3028)	15.55 ± 0.35	15207.9	36	4	3.89	*Pisum*
A. arbuscula subsp. *longicaulis* (2855)*	22.85 ± 0.18	22347.3	54	6	3.81	*Pisum*
A. arbuscula subsp. *longicaulis* (2860)*	23.10 ± 0.39	22591.8	54	6	3.85	*Petunia*
A. arbuscula subsp. *thermophila* (3032)*	9.47 ± 0.13	9261.66	18	2	4.73	*Pisum*
A. argillosa (3034)*	15.77 ± 0.65	15423.06	36	4	3.94	*Petunia*
A. bigelovii (3051)	8.00 ± 0.10	7824.00	18	2	4.00	*Petunia*
A. bigelovii (3050)	15.06 ± 0.13	14728.68	36	4	3.76	*Pisum*
A. bigelovii (2869)	15.32 ± 0.09	14982.96	36	4	3.83	*Pisum*
A. cana subsp. *bolanderi* (3047)*	9.01 ± 0.09	8811.78	18	2	4.50	*Petunia*
A. cana subsp. *cana* (2128)	27.04 ± 0.42	26445.12	72	8	3.38	*Pisum*
A. cana subsp. *viscidula* (2844)	8.73 ± 0.24	8537.94	18	2	4.37	*Petunia*
A. cana subsp. *viscidula* (2851)	8.51 ± 0.13	8322.78	18	2	4.26	*Petunia*
A. cana subsp. *viscidula* (2875)	8.58 ± 0.19	8391.24	18	2	4.29	*Petunia*

A. longiloba (3025)*	16.62 ± 0.45	16254.36	36	4	4.15	*Pisum*
A. nova (3053)	9.09 ± 0.06	8890.02	18	2	4.51	*Petunia*
A. nova (2873)	17.25 ± 0.15	16870.5	36	4	4.31	*Pisum*
A. nova (2876)	17.10 ± 0.11	16723.8	36	4	4.28	*Pisum*
A. nova var. *duchesnicola* (3029)*	22.90 ± 0.39	22396.2	54	6	3.82	*Pisum*
A. nova var. *duchesnicola* (3030)*	22.43 ± 0.24	21936.54	54	6	3.74	*Pisum*
A. pygmaea (2836)	10.89 ± 0.24	10650.42	18	2	5.45	*Petunia*
A. pygmaea (2870)	11.14 ± 0.19	10894.92	18	2	5.57	*Petunia*
A. rigida (2859)*	8.23 ± 0.13	8048.94	18	2	4.12	*Petunia*
A. rothrockii (19803)*	16.41 ± 0.25	16048.98	36	4	4.10	*Pisum*
A. tridentata subsp. *parishii* (3037)*	16.61 ± 0.27	16244.58	36	4	4.15	*Pisum*
A. tridentata subsp. *parishii* (3038)*	16.32 ± 0.17	15960.96	36	4	4.08	*Pisum*
A. tridentata subsp. *spiciformis* (2839)	9.00 ± 0.19	8802	18	2	4.50	*Petunia*
A. tridentata subsp. *tridentata* (1996)	8.42 ± 0.27	8234.76	18	2	4.21	*Petunia*
A. tridentata subsp. *tridentata* (2871)	8.24 ± 0.25	8058.72	18	2	4.12	*Petunia*
A. tridentata subsp. *vaseyana* (2879)	15.12 ± 0.37	14787.36	36	4	3.78	*Petunia*
A. tridentata subsp. *vaseyana* (2872)	8.89 ± 0.20	8694.42	18	2	4.45	*Petunia*
A. tridentata subsp. *vaseyana* (2874)	8.85 ± 0.22	8655.3	18	2	4.43	*Petunia*
A. tridentata subsp. *wyomingensis* (2880)*	15.07 ± 0.19	14738.46	36	4	3.77	*Petunia*
A. tridentata subsp. *xericensis* (2858)*	16.24 ± 0.13	15882.72	36	4	4.06	*Pisum*
A. triparitita subsp. *rupicola* (3033)*	8.68 ± 0.19	8489.04	18	2	4.34	*Petunia*
A. tripartita subsp. *tripartita* (3054)	8.85 ± 0.08	8655.30	18	2	4.42	*Petunia*
A. tripartita subsp. *tripartita* (2845)*	15.32 ± 0.18	14982.96	36	4	3.83	*Petunia*
Hybrids						
A. cana subsp. *cana* x *A. tridentata*	19.15 ± 0.68	18728.70	54	6	3.19	*Pisum*

subsp. *wyomingensis* (2759)						
A. cana subsp. *cana* x *A. tridentata* subsp. *wyomingensis* (2760)	18.72 ± 0.35	18308.16	54	6	3.12	*Pisum*
A. tridentata subsp. *tridentata* x *A. tridentata* subsp. *vaseyana* (3048)	15.71 ± 0.14	15364.38	36	4	3.93	*Pisum*
A. tridentata subsp. *tridentata* x *A. tridentata* subsp. *vaseyana* (3049)	8.52 ± 0.25	8332.56	18	2	4.26	*Petunia*
Outgroup species						
A. californica (3039)*	8.38 ± 0.22	8195.64	18	2	4.19	*Petunia*
A. californica (3043)*	8.57 ± 0.12	8381.46	18	2	4.28	*Petunia*
A. filifolia (2868)	7.26 ± 0.06	7100.28	18	2	3.63	*Petunia*
A. ludoviciana (3087)*	13.82 ± 0.17	13515.95	36	4	3.45	*Pisum*
A. palmeri (3044)*	7.14 ± 0.07	6982.92	18	2	3.57	*Pisum*
A. papposa (3077)*	8.44 ± 0.17	8254.32	18	2	4.22	*Petunia*

Note: The taxa for which genome size has been estimated for the first time are marked with an asterisk (*). [1]2C nuclear DNA content (mean value ± standard deviation of 10 samples). [2]1 pg = 978 Mbp (Doležel et al., 2003). [3]Somatic chromosome number. [4]Monoploid genome size (2C value divided by ploidy level, Greilhuber et al., 2005). All chromosome counts have been carried out in the populations studied in the present paper. [5]Internal standard used in each case (see text for details about *Pisum* and *Petunia*).

TABLE 3. Previous results.

Taxa	2C ± s.d. (pg)	2C (Mbp)	2n	Ploidy level	2C/P.l.	Standard
A. arbuscula subsp. *arbuscula*[2]	9.22± 0.11	9017.16	18	2	4.61	*Petunia*
A. bigelovii[2]	15.49 ± 0.10	15149.22	36	4	3.87	*Pisum*
A. cana subsp. *cana*[1]	25.65 ± 0.61	25085.70	72	8	2.57	*Pisum*
A. cana subsp. *viscidula*[2]	8.54 ± 0.09	8352.12	18	2	4.27	*Petunia*
A. nova[2]	6.37 ± 0.14	6229.86	18	2	3.19	*Petunia*
A. pygmaea[2]	11.54 ± 0.18	11286.12	18	2	5.77	*Petunia*
A. tridentata subsp. *spiciformis*[1]	8.18 ± 0.30	8000.04	18	2	4.09	*Pisum*
A. tridentata subsp. *tridentata*[2]	8.17 ± 0.08	7990.26	18	2	4.09	*Petunia*
A. tridentata subsp. *vaseyana*[2]	8.66 ± 0.07	8469.48	18	2	4.33	*Petunia*

Note: The data belong to previous studies in nuclear DNA amount in the genus *Artemisia*: [1] Torrell and Vallès, 2001; [2]Garcia et al., 2004. Data of the *A. nova* population is not at all consistent with those of the five populations studied in the present paper, so that we believe that it could be the product of a misidentification.

TABLE 4. Environmental, ecological and morphological characteristics of the diploid *Artemisia* of this study.

Taxa*	Subg.[1]	2C[2]	Dist.[3]	Alt. range[4]	Mean ann. prec.[5]	Plant height[6]	Seed prod.[7]	Cap. rec. aft. burn.[8]	Growth rate[9]	Veg. reprod.[10]	Drought tol.[11]	Salinity tol.[12]
A. arbuscula subsp. *arbuscula*	T	9.16	4	5	3	1	1	2	1	N	1	1
A. arbuscula subsp. *termophila*	T	9.47	2	2	3	1	1	1	1	N	1	2
A. bigelovii	T	8	2	4	2	2	4	1	2	N	3	2
A. cana subsp. *bolanderi*	T	9.01	2	4	3	2	4	3	2	Y	1	1
A. cana subsp. *viscidula*	T	8.59	4	5	3	3	3	3	3	Y	1	1
A. nova	T	9.09	4	5	1	2	3	1	2	N	3	2
A. pygmaea	T	11.19	2	2	1	1	2	1	1	N	3	2
A. rigida	T	8.23	2	4	2	1	1	2	1	N	3	2
A. tridentata subsp.	T	8.59	3	5	3	3	3	2	3	Y	1	1

spiciformis												
A. tridentata subsp. *tridentata*	T	8.28	5	4	3	4	4	3	3	N	2	1
A. tridentata subsp. *vaseyana*	T	8.8	5	5	1	4	4	1	2	N	2	1
A. tripartita subsp. *rupicola*	T	8.68	2	3	2	1	3	1	1	Y	1	2
A. tripartita subsp. *tripartita*	T	8.85	3	3	3	3	3	1	2	Y	2	2
A. californica	O	8.47	2	3	2	3	3	1	2	Y	2	1
A. filifolia	O	7.26	5	-	3	3	4	3	3	Y	2	3
A. palmeri	O	7.14	1	-	2	4	4	2	3	N	2	1
A. papposa	O	8.44	1	-	2	1	2	1	1	N	2	2

*Only diploid taxa have been considered for this analysis in order not to bias the results due to the effect of genome size downsizing in polyploids.

[1]Subgenus. T= *Tridentatae*, O= Outgroup (other subgenera).

[2]Nuclear DNA amount 2C value. Mean values of the populations measured in the present work and in the previous ones (Torrell and Valles, 2001; Garcia et al. 2004). The result given for *A. nova* in Garcia et al. (2004) has not been considered (see Table 3).

[3]Distribution. Values from 1= restricted, to 5= general.

[4]Altitude range. Values from 1= narrow, to 5= large.

[5]Mean annual precipitation. Values from 1= lower, to 5= higher.

[6]Plant height. Values from 1= smaller, to 5= taller.

[7]Seed production. Values from 1= low seed producer, to 4 = high seed producer.

[8]Capacity of recover after burning. Values from 1= essentially no or very slow recovery, to 3 = better capacity of recovering after burning.

[9]Growth rate. Values from 1= slower, to 3 = faster.

[10] Vegetative reproduction, where Y = sometimes reproduces vegetatively and N= never reproduces vegetatively

11 Drought tolerance. Values from 1= bad drought tolerant to 3 = better drought tolerant.

[12] Salinity tolerance. Values from 1= bad salinity tolerant to 3 = better salinity tolerant.

Fig. 1. Genome sizes (2C) of the hybrids and their parental species.

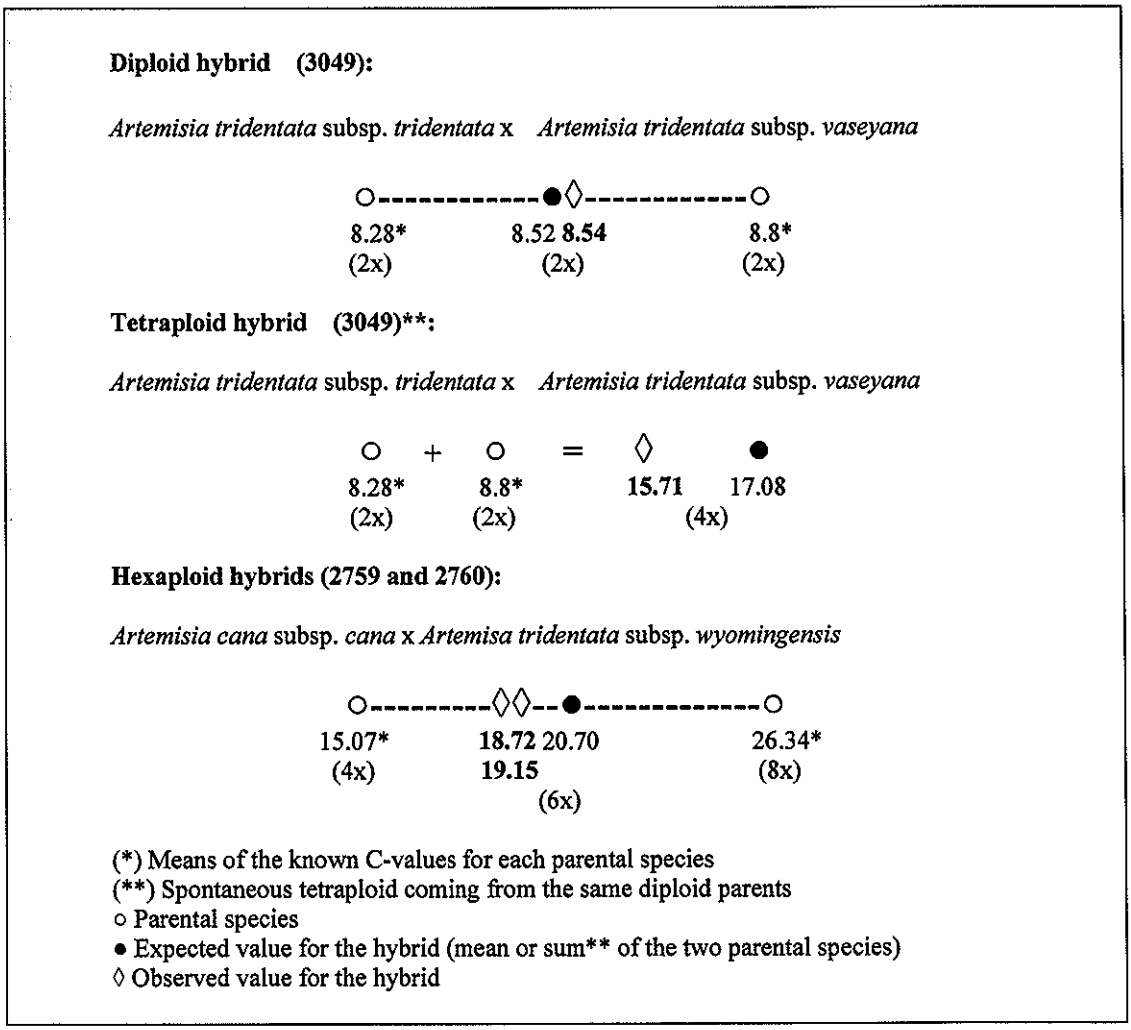


Fig. 2. Cluster analysis using mean 2C values for the diploid *Tridentatae* species (Nearest neighbour method, square euclidean). The numbers represent the euclidean distance.

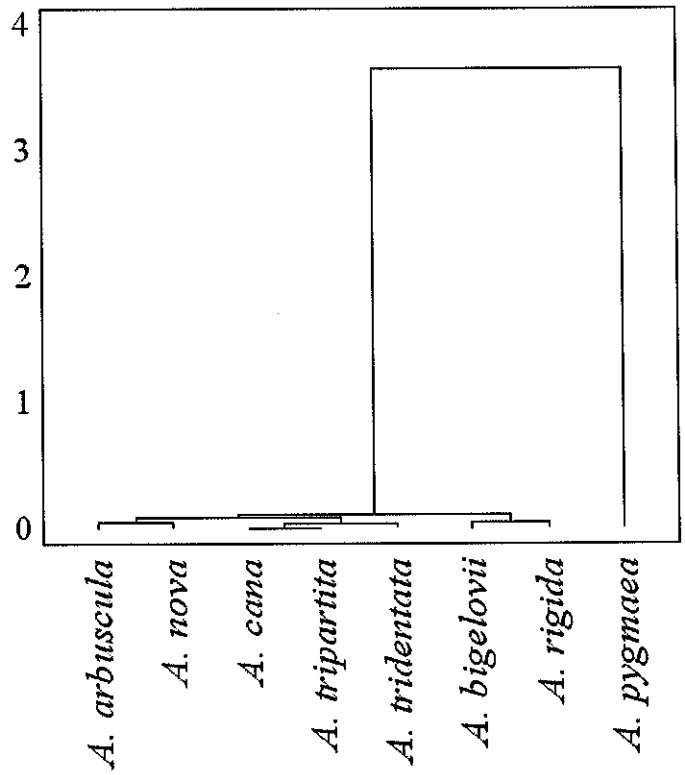


Fig. 1.

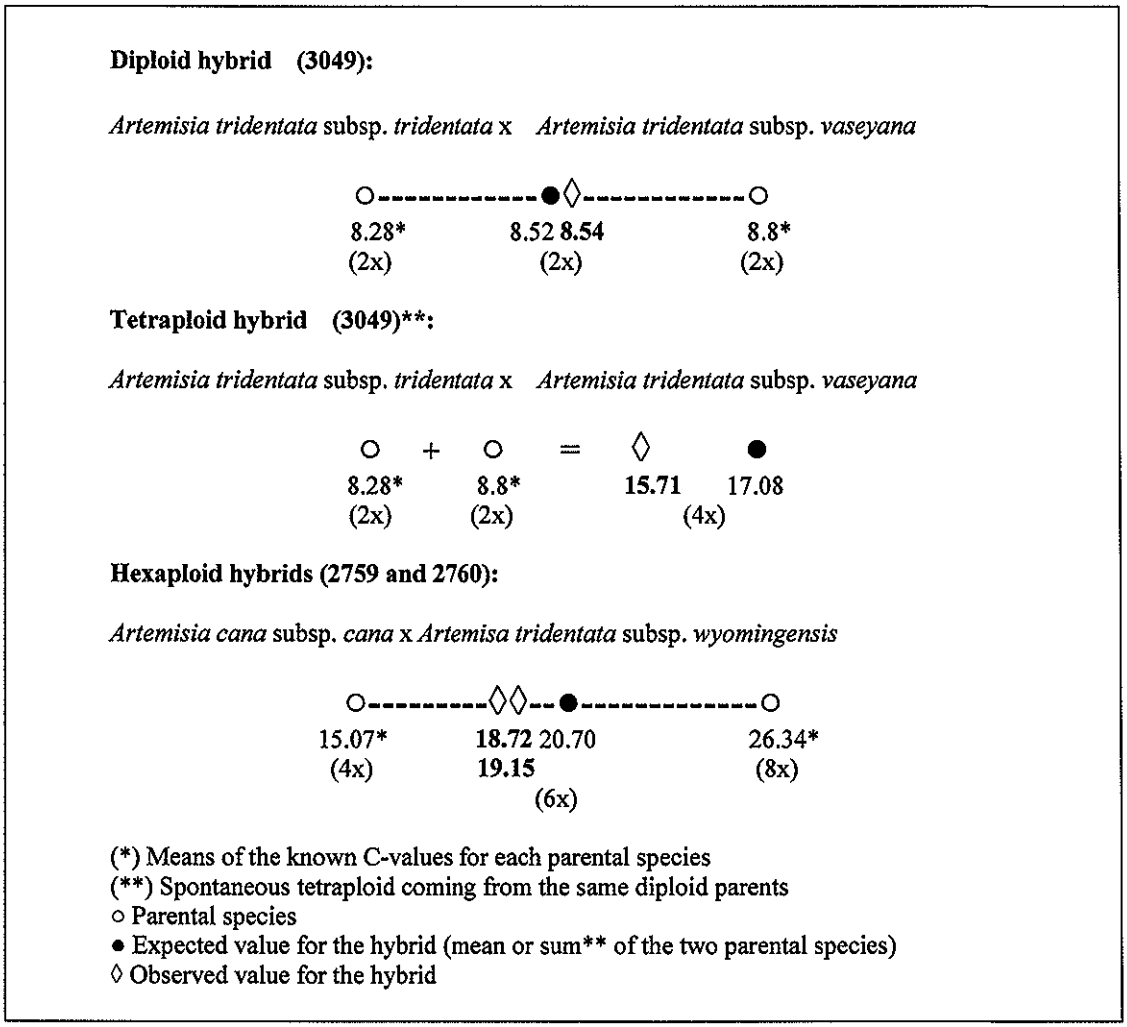
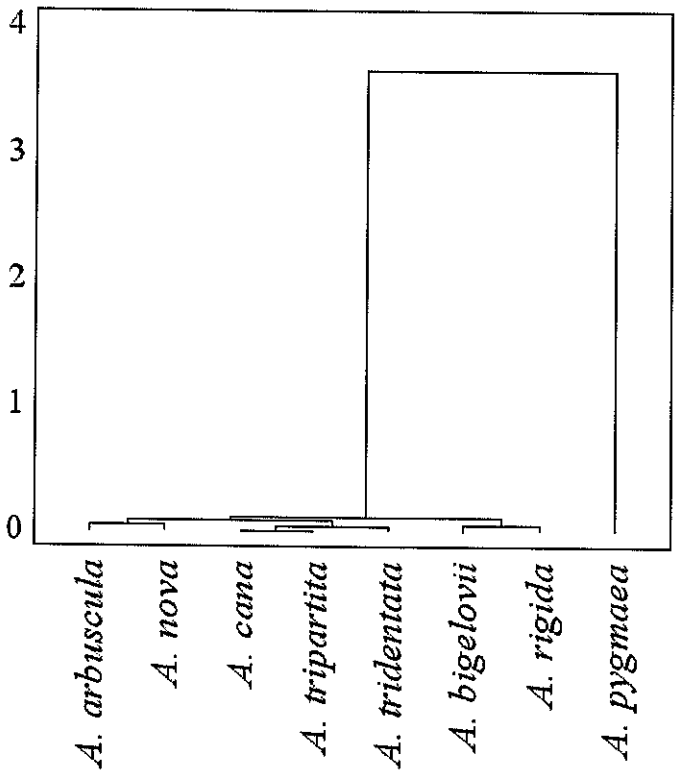

Diploid hybrid (3049):
Artemisia tridentata subsp. *tridentata* x *Artemisia tridentata* subsp. *vaseyana*
○----------●◊----------○
8.28* 8.52 **8.54** 8.8*
(2x) (2x) (2x)
Tetraploid hybrid (3049):**
Artemisia tridentata subsp. *tridentata* x *Artemisia tridentata* subsp. *vaseyana*
○ + ○ = ◊ ●
8.28* 8.8* **15.71** 17.08
(2x) (2x) (4x)
Hexaploid hybrids (2759 and 2760):
Artemisia cana subsp. *cana* x *Artemisa tridentata* subsp. *wyomingensis*
○---------◊◊--●----------○
15.07* **18.72** 20.70 26.34*
(4x) **19.15** (8x)
(6x)
(*) Means of the known C-values for each parental species
(**) Spontaneous tetraploid coming from the same diploid parents
○ Parental species
● Expected value for the hybrid (mean or sum** of the two parental species)
◊ Observed value for the hybrid

Fig. 2.


4
3
2
1
0
A. arbuscula
A. nova
A. cana
A. tripartita
A. tridentata
A. bigelovii
A. rigida
A. pygmaea